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ENERGY TRANSDUCTION IN QUINONE INHIBITION OF INSECT FEEDING*

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Abstract—Variously substituted 1,4-naphthoquinones inhibited (deterred) feeding by *Periplaneta americana*. Certain sensilla on the antennae were important receptors for the deterrent stimulus. Dendritic branches of sensory neurons in the sensilla are exposed to the exogenous inhibitory chemicals via pores which penetrate the cuticle. The order of relative degree of complexing of a given naphthoquinone to density-gradient fractions of antennal homogenates rich in nerve membrane fragments matched the order of relative deterrency of that chemical to feeding. Sulphhydryl groups of protein in the antennae were important sites of reaction. Energy transduction at the receptor site involved complexing of the quinone with the receptor chemical and the ultimate reduction of the quinone to its quinol. Energy transfer between sulphhydryl groups and quinones provided a mechanism which could bring about a change in conformation of the receptor macromolecule which could allow inorganic ion flows to generate the action potential of the neuron.

INTRODUCTION

CHEMORECEPTION by insects, whether it involves pheromones, host-derived attractants, gustatory stimuli, deterrents, and/or repellents, etc., necessitates the exchange of energy between the chemical messenger and the receptor neuron. Interpretation of such energy-transduction mechanisms in animals has been only theoretical (AMOORE, 1965), but NORRIS (1969, 1970) recently presented biological and chemical data to support a quinol–quinone system as such a mechanism in the chemoreception of the beetle, *Scolytus multistriatus* (Marsh.). The possible rôle of certain quinol–quinones in chemoreception by the cockroach, *Periplaneta americana*, has been variously studied, and portions of our findings are presented in this paper.

MATERIALS AND METHODS

Bioassay of feeding

The relative feeding inhibition (deterrency) of a group of 1,4-naphthoquinones to starved *P. americana* adults was investigated in behavioural assays. Because

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P. americana is a rather general feeder, it was necessary to establish a definable feeding stimulus to which the naphthoquinones could be readily added. A chloroform extract of crushed dog biscuits (Purina Co.), a food commonly used in the laboratory culture of cockroaches, proved to be a suitable positive gustatory stimulus. The naphthoquinone and/or biscuit extract was bioassayed by adsorbing the solutions into pressed cellulose tablets (5 mm thick × 20 mm dia.) which were formed from a 10 : 1 (w/w) mixture of powdered and fibrous cellulose. Each previously weighed tablet was submerged for 1 hr in either (1) 15 ml of the biscuit extract (1 ml equivalent to 2·66 g of dried crushed biscuits) or (2) 0·01 M naphthoquinone in the biscuit extract, then removed, air and oven dried, and weighed. One extract-treated and one extract + naphthoquinone-treated tablet were placed in separate 1·25 oz. paper (portion-control) cups (American Paper & Plastics Products, Inc.) which were glued, in diametrically opposed positions, to the bottom of a 1 gal cardboard container. Four male *P. americana* adults, which had been starved for 2 weeks, were enclosed in each container and so held for 3 days in darkness at 27°C. There were five replicates per treatment. After the 3 days, the tablets were removed, oven-dried, and weighed. The relative weight loss of the tablets was used as a measure of the feeding inhibition of a naphthoquinone treatment.

ROTH and WILLIS (1950) demonstrated that detection of the female-produced sex pheromone by male cockroaches was via the antennae. However, the locations of receptors involved in cockroach feeding behaviour have not been investigated adequately. To better locate chemoreceptors important to gustation by *P. americana*, the following deletion experiments were performed. Groups of adult males were partially or wholly antennectomized and/or maxillectomized in several combinations, and after a 24-hr recuperative period they were given a choice of tablets treated with biscuit extract vs. solvent. Treatments were: (1) control insects, no deletions; (2) one antenna removed; (3) both antennae removed; (4) both maxillary palps removed; and (5) both antennae and both maxillary palps removed. There were five replications of 4 cockroaches/treatment.

The aforementioned experiment was repeated except (1) in treatment 5 both maxillary and labial palps were removed and the antennae were left intact; and (2) the choice of chemical treatments was biscuit extract (positive control) vs. 0·01 M 1,4-naphthoquinone in the extract.

The external morphology and ultrastructure of chemoreceptor sensilla on the antennae of *P. americana* were studied by scanning- and transmitting-electron microscopy (BORG and NORRIS, 1971).

Preparation of nerve cell fractions

Antennae from both male and female *P. americana* adults were excised at the base, and immediately placed in 5 ml of ice cold 0·25 M sucrose and homogenized in a motor-driven Teflon-glass homogenizer. The homogenation and fractionation methods were patterned after DE ROBERTIS (1961) and TELFORD and MATSUMURA (1970). The homogenate was filtered through glass wool into a 1 × 3 in.

centrifuge tube, and centrifuged at 20,000 *g* for 45 min. After decanting, the pellet was twice resuspended, washed, and centrifuged with 5 ml of 0·25 M sucrose. The final pellet was taken up in 6 ml of 0·8 M sucrose and layered onto a discontinuous sucrose density-gradient composed of 6 ml each of 1·0, 1·2, 1·5, and 1·8 M sucrose. The gradient tubes were placed in a SW 25·1 rotor and centrifuged at 90,000 *g* (max) for 120 min. The particulate band located at each density interface was removed with a 5 ml syringe, diluted to approximately 0·25 M with H_2O, and repelleted at 20,000 *g* for 45 min. Beginning with the band at the 0·8 to 1·0 M interface and ending with the band at the 1·5 to 1·8 M interface, the bands were designated F_1 through F_4. The protein content of an aliquot of each band was determined by the method of LOWRY *et al.* (1951). Another aliquot of each band was fixed in glutaraldehyde, post-fixed in osmium, dehydrated in acetone, embedded in Araldite, stained with uranyl acetate and lead citrate, and examined by electron microscopy.

Investigation of complexing between 2-(*methyl*-C^{14})-1,4-*naphthoquinone and particulate bands from antennal homogenate*

To determine the binding affinity of the feeding deterrent, 2-(methyl-C^{14})-1,4-naphthoquinone, for particulate material in the density-gradient bands F_{1-4} from the antennal homogenate, the following procedure was used. The antennal homogenate was prepared as previously described, but before the centrifugation procedure it was incubated for 15 min at room temperature with 0·1 μc of 2-(methyl-C^{14})-1,4-naphthoquinone (final concentration was 2×10^{-6} M). The incubate was centrifuged at 20,000 *g*, and the pellet obtained was resuspended in 0·25 M sucrose and recentrifuged three times to remove radioactivity not bound to particulate matter. Aliquots of each wash were analysed for radioactivity. The final pellet obtained was fractionated on the density gradient as described previously. Samples for counting were placed in 10 ml of methyl cellusolve-based counting solution (toluene, 500 ml; methyl cellusolve, 500 ml; PPO, 5·5 g; and POPOP, 300 mg) and counted in a Packard 3380 liquid-scintillation counter. Each sample vial was counted twice for 10 min and the count was averaged. Quenching was monitored with an external standard.

Ultra-violet spectroscopy of binding of naphthoquinones to components of the supernatant and particulate fractions of the antennal homogenate

Three hundred pairs of *P. americana* antennae were homogenized in a Potter–Elvehjem homogenizer containing 23 ml of ice cold 0·05 M Tris buffer, pH 7·0. The homogenate was filtered through glass wool to remove cuticular debris and rinsed with 15 ml of Tris. The homogenate then was subjected to the same centrifugation procedure as described for the radiolabelled naphthoquinone-binding study. The initial decantate (supernatant) from the centrifugation procedure was saved for study of the complexing (binding) of its soluble and particulate components, not sedimented at 20,000 *g*, with deterrent naphthoquinone. Bands removed from the sucrose gradient were diluted with Tris to 50 ml, and

then centrifuged at 32,800 *g* for 2 hr. The resultant pellets were each resuspended in 4 ml of Tris.

Ultra-violet spectroscopy methods patterned after DASTOLI and PRICE (1966) and DONOVAN (1969) were used to determine the relative binding affinity of the particulate fractions and decantate for the feeding deterrents, 2-methyl-1,4-naphthoquinone, 1,4-naphthoquinone, and 5-hydroxy-1,4-naphthoquinone. Absorption measurements were made with a Cary Model 15 spectrophotometer using a 0·0 to 0·1 scale expansion. The dynode setting was 2, and the amplifier sensitivity was 2. Absorption cells with a path length of 1 cm were used. Baselines (zero absorbance as a function of wavelength) were determined with an aliquot of a fraction or the decantate in both the sample and reference cells. The four particulate fractions (bands F_{1-4}) and the decantate were tested at concentrations producing an optical density of 0·2 at 280 nm. These concentrations were obtained by diluting each aliquot with Tris. Enough naphthoquinone (0·13 mM solution in de-ionized water) was added to the solution of the aliquot in the sample cell to yield a final concentration of $1{\cdot}7 \times 10^{-7}$ M naphthoquinone. The decrease in optical density induced at 280 nm was then recorded at time zero. Corrections for absorption of the naphthoquinone at 280 nm were made by subtracting the absorbance of each chemical alone in Tris from the absorption value obtained with the naphthoquinone in combination with the antennal fraction. All naphthoquinones used were recrystallized from redistilled benzene.

As stated by SZENT-GYÖRGYI (1960), in energy transfer two events are to be considered: (1) the extent of coming together (complexing) of involved molecules, and (2) the actual transfer of energy. To further investigate the extent of complexing of deterrent quinone with receptor sites (i.e. chemicals) in aliquots from the antennal preparation, or with model receptor chemicals (i.e. cysteine or reduced glutathione), a deterrent to insect gustation, *p*-benzoquinone (NORRIS, 1970) or 1,4-naphthoquinone, was added to aliquots of F_2 or decantate, or to cysteine or reduced glutathione, in buffer solution, and each mixture was frozen in a covered glass container. This is one method of bringing the candidate molecules more closely together. When frozen, a preparation was removed and observed microscopically for a change of colour from those of the frozen control solutions of the individual test compounds. In some cases, after melting, the preparation was scanned in the spectrophotometer for changes in absorbance. This analysis was aimed at determining whether an energy-transfer reaction had occurred between complexing components.

Dropping-mercury-electrode polarography of complexing in model systems

In the absence of sufficiently purified quantities of receptor chemical from antennae for polarographic study of energy-transfer complexing with quinones, the interaction between a model receptor chemical (i.e. reduced glutathione) and quinones with or without a sulphhydryl group-inhibitor chemical (N-ethyl maleimide) was investigated. Reduced glutathione was chosen as a model receptor-site chemical because the complexing and/or oxidative reactions of quinones with

sulphhydryl groups of proteins are documented (WEBB, 1966), and the sulphhydryl-containing, reduced glutathione is an important constituent of neural tissues (PISANO, 1969).

The solutions to be polarographed were prepared as follows: (1) 100 ml of 0·1 M phosphate buffer, pH 7, were deoxygenated in a covered glass system for 25 min with prepurified nitrogen; (2) then the weighed amount of test chemical(s) was added to the buffer solution; (3) nitrogen was bubbled through the preparation for 15 min; (4) avoiding unnecessary contact with air, 20 ml of the preparation were transferred to a proper glass vessel for polarizing; (5) nitrogen was passed through the solution in the vessel for 15 min; and (6) with the nitrogen stream removed from the test solution, but with a thin stream of nitrogen running across the surface of the liquid, the polarographic analyses were run. All studies were conducted using a Type PO4 Radiometer Polariter with the dropping-mercury electrode.

RESULTS AND DISCUSSION

Bioassay of feeding

Based on the weight loss of assay tablets due to feeding by cockroaches, all tested naphthoquinones were inhibitory to feeding (Fig. 1). The relative inhibition at 0·01 M concentration was 5-hydroxy-1,4-naphthoquinone ≥ 1,4-naphthoquinone > 2-hydroxy-1,4-naphthoquinone > 2-methyl-1,4-naphthoquinone. It is interesting that this same order of relative inhibition to feeding by the bark beetle, *S. multistriatus*, was found by NORRIS (1969, 1970).

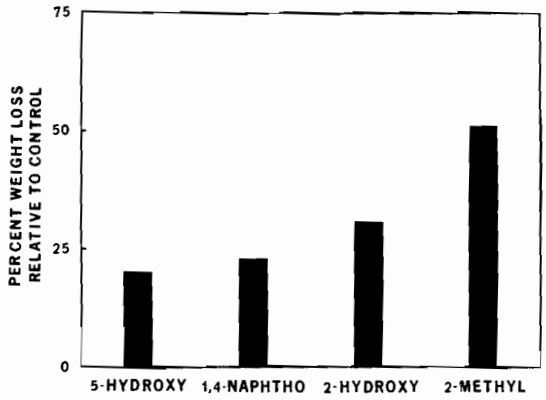

FIG. 1. The relative feeding inhibition of a group of 1,4-naphthoquinones to starved male *P. americana* adults as investigated in behavioural bioassays. Weight loss was that of treated vs. positive control cellulose tablets attributable to feeding by the insects.

Differences in the amount of feeding by cockroaches which were partially or wholly antennectomized and/or maxillectomized in several combinations (Fig. 2)

indicated that cockroaches with one or both antennae, with or without maxillary palps, were stimulated to feed by the extract from biscuits. Removal of both antennae, or both antennae and both maxillary palpi, prevented a significant feeding response to the gustatory stimulus (Fig. 2). Though the degrees of trauma associated with the latter two treatments complicate interpretation of the results, the findings from these total deletion studies would indicate that the antennae are important to chemoreception related to gustation by *P. americana*.

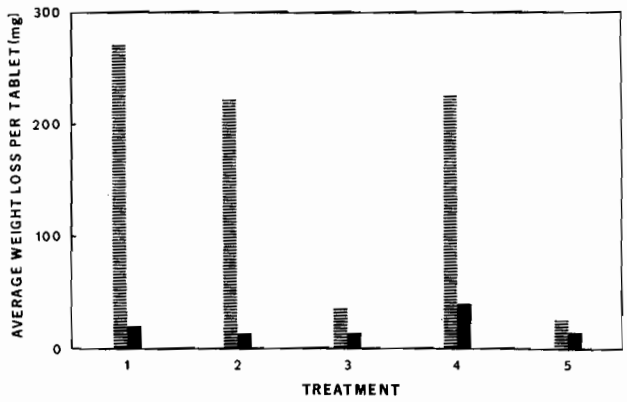

FIG. 2. Effects on feeding of partially or wholly antennectomizing and/or maxillectomizing male *Periplaneta americana* adults. Solid black bars represent feeding on solvent-treated (control) tablets and the striped bars present feeding on biscuit extract-treated tablets. Specific five treatments are given in the text.

In the second deletion study (Fig. 3), the cockroaches fed significantly more on the positive control (i.e. biscuit extract) than on the extract + the inhibitor (i.e. 1,4-naphthoquinone), except when both antennae were removed. These results also indicate that the antennae are important to gustation by cockroaches. If the antennae were present, there was still significantly more feeding on the positive-control than the inhibitor-added tablets even with the maxillary and labial palps removed.

Electron microscopy of chemoreceptor sensilla on the antennae of the cockroach (BORG and NORRIS, 1971) indicated that dendritic branches of sensory neurons in such sensilla are exposed to the external environment as found by BORG and NORRIS (1970) for *S. multistriatus*.

Complexing between 2-(methyl-C^{14})-1,4-naphthoquinone and particulate bands from antennal homogenates

Of the recovered radioactivity, which was 63% of the original, 84% was found in the soluble fraction and 16% was in the particulate fractions (Table 1)

from the sucrose-density gradient. Because chemoreception in gustation and olfaction must by its nature be a transient phenomenon, we expected a low level of deterrent binding to the particulate fractions. In addition, 2-methyl-1,4-naphthoquinone is one of the less effective tested naphthoquinone deterrents,

FIG. 3. Effects on detection of naphthoquinone deterrent to feeding of partially or wholly antennectomizing, maxillectomizing, and/or labialectomizing male *P. americana* adults. Solid black bars represent feeding on biscuit extract-treated tablets, and the striped bars represent feeding on naphthoquinone + biscuit extract-treated tablets. Specific five treatments are given in the text.

TABLE 1—RADIOACTIVITY BOUND IN PARTICULATE FRACTIONS ISOLATED ON A SUCROSE-DENSITY GRADIENT AFTER THE ANTENNAL HOMOGENATE WAS INCUBATED WITH 0·1 μc OF 2-(METHYL-C^{14})-1,4-NAPHTHOQUINONE

Fraction	Total protein (μg)	Total counts/min	Counts/min per mg protein
F1	314	2431	7742
F2	157	1270	8089
F3	1447	8272	5716
F4	1078	4840	4489
Residue	1072	6105	5694

so the extent of binding perhaps should not be as great as with 5-hydroxy-1,4-naphthoquinone. However, 2-methyl-1,4-napthoquinone was the only radio-labelled naphthoquinone available to us. The radioactivity found in each fraction (band) is shown in Table 1. The specific activity per mg of protein was greatest in bands F_1 and F_2. Electron micrographs of aliquots of these particulate fractions from the antennal homogenate indicated the presence of nerve membrane fragments and vesicles.

Ultra-violet spectroscopy of binding of naphthoquinones to components of the antennal homogenate

The change in u.v. absorbance at 280 nm induced by each naphthoquinone interacting with components of each particulate fraction (i.e. F_{1-4}) or the decantate from the antennal homogenate is shown in Fig. 4. The greatest change in absorbance with each naphthoquinone occurred with fraction F_2. Absorbance with F_1 was intermediate; and that with F_3, F_4 and the decantate was similar, and

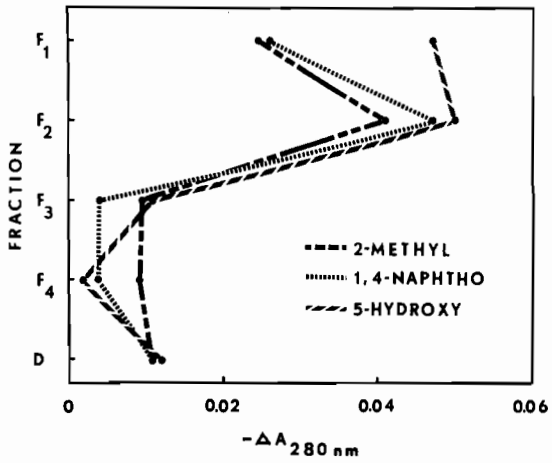

FIG. 4. Differential absorbance at 280 nm upon addition of the given naphthoquinone ($1{\cdot}7 \times 10^{-7}$ M) to the sample cell. The sample had been previously balanced against the reference with a concentration of the given fraction or the decantate (D) which produced an optical density of 0·2 units.

significantly less than with F_1. Considering the relative change in absorbance at 280 nm associated with each naphthoquinone interacting with a given fraction or the decantate, 5-hydroxy-1,4-naphthoquinone yielded the largest absorbance change, 1,4-naphthoquinone was intermediate, and 2-methyl-1,4-naphthoquinone gave the least with fractions F_{1-3} and the decantate. With fraction F_4, the pattern was reversed.

It is assumed that the magnitude of change in absorbance is directly related to the degree of binding of a naphthoquinone with components of a particulate fraction or the decantate. Because in these experiments, both the reference cell and the sample cell contained a solution of one of the particulate fractions or the decantate, any change in the conformation of components in a solution absorbing at 280 nm after addition of the naphthoquinone to the sample cell was reflected as a change in absorbance relative to that of the solution in the reference cell.

These total experiments established that the order of relative deterrency of three naphthoquinones bioassayed with *P. americana* was the same as their order

of relative degree of binding with antennal homogenate fractions F_1 and F_2 which especially contained nerve membrane fragments. Because dendritic branches of chemosensory neurons are naturally exposed to the external environment (i.e. the source of chemical stimulants and deterrents to gustation), the above correlation between *in vivo* bioassay and *in vitro* binding data is considered indicative that the type of complexing observed between naphthoquinones and components of F_1 and F_2 involves a natural energy-transduction mechanism for inhibition of gustation by *P. americana*. The relative importance of components of F_1 and F_2, as compared to F_3 and F_4, as binding (reaction) sites for chemoreception by *P. americana* was further supported by the higher degrees of binding of the radioactivity of the deterrent, (2-methyl-C^{14})-1,4-naphthoquinone, to antennal fractions F_1 and F_2 than F_3 and F_4.

Our experimental findings do not completely clarify the energy-transduction mechanism involved in the quinone inhibition of gustation by *P. americana*, or *S. multistriatus* (NORRIS, 1969, 1970); however, our results indicate that sulphhydryl groups in receptor protein of the sensory neurons are reaction sites. Quinones participate in electron transport and oxidative phosphorylation (MORTON, 1965), behave chemically like alpha-beta unsaturated ketones, and may react with thiols by 1,4 addition (MORRISON *et al.*, 1969) and/or by oxidation (WEBB, 1966). Our studies employing spectrophotometric analysis of the interactions between deterrent quinones and components of the antennal homogenate at 27°C proved that binding (i.e. complexing) occurred, but these analyses as run at zero time failed to reveal the nature of any reaction products. In an attempt to intensify the energy-transfer reaction between quinone and an aliquot of the decantate or fraction F_2 so reaction products might then be qualitatively detected by u.v. spectrophotometry, the mixture of quinone and aliquot was frozen, thawed, and then immediately analysed by u.v. This study showed that the interaction of the quinone and given aliquot produced a new u.v. absorbance peak which was caused by the corresponding quinol (e.g. a new peak at 290 nm attributable to hydroquinone, appeared after freezing *p*-benzoquinone with an aliquot of F_2). Thus, in these experimental conditions, the inhibitory quinone was reduced. Colour-change indications of energy-transfer complexing between quinone feeding deterrents and aliquots of certain antennal fractions, or given concentrations of model receptor chemicals (i.e. sulphhydryl-containing cysteine or reduced glutathione) upon freezing are shown in Table 2. The interactions of (1) 10^{-3} or 5×10^{-4} M 1,4-naphthoquinone with 10^{-3} or 5×10^{-4} M glutathione or cysteine and (2) 10^{-3} M 1,4-naphthoquinone with a given aliquot of antennal components produced characteristic colour changes (Table 2). When 10^{-3} or 5×10^{-4} M 1,4-naphthoquinone was frozen with 10^{-3} or 5×10^{-4} M glutathione or cysteine + 10^{-3} or 5×10^{-4} M N-ethyl maleimide (a sulphhydryl-group inhibitor) the colour produced was different from that produced by the naphthoquinone alone with glutathione or cysteine. According to SZENT-GYÖRGYI (1960), such a difference in colour would be attributable to competitive complexing by 1,4-naphthoquinone and N-ethyl maleimide with the glutathione or cysteine. Colour-change indications of such complexing between

TABLE 2—COLOUR CHANGE INDICATIONS OF THE FORMATION OF AN ENERGY-TRANSFER COMPLEX BETWEEN GIVEN CHEMICALS WHEN QUICK FROZEN IN A BUFFER SOLUTION

Quinone (M)	Reduced glutathione				Glutathione + N-ethyl maleimide		Cysteine		Cysteine + N-ethyl maleimide		Antennal components	
	10^{-2}	10^{-3}	5×10^{-4}	10^{-4}	10^{-3}*	5×10^{-4}*	10^{-2}	5×10^{-4}	$5\times10^{-4}+10^{-3}$	5×10^{-4}*	Decantate	F_2
1,4-Naphthoquinone:												
10^{-3}		++			+						++	++
5×10^{-4}			++			+		++		+		
p-Benzoquinone:												
10^{-2}	++	++					++	++	+		++	++
10^{-3}	−	++		−								
5×10^{-4}			++			+						
10^{-4}		−										
10^{-5}		−										

* Both chemicals mixed with the given quinone were at this molar concentration; ++, a strong change in colour indicative of energy-transfer complexing; +, a colour change different from ++, but characteristic of competitive formation of energy-transfer complexes between the indicated sulphhydryl-containing chemical and the given quinone or N-ethyl maleimide; −, combinations tested which yielded no colour change indicative of complexing.

various concentrations of *p*-benzoquinone and aliquots of antennal components, or given concentrations of glutathione or cysteine, when frozen, also were obtained (Table 2). When both 5×10^{-4} M *p*-benzoquinone and 5×10^{-4} M N-ethyl maleimide were frozen with 5×10^{-4} M glutathione or cysteine a colour change distinctly different from that produced by the quinone alone with glutathione or cysteine also was observed (Table 2). Thus, N-ethyl maleimide also competed with *p*-benzoquinone, apparently for the thiols on the glutathione or cysteine.

Our polarographic investigations of interactions among feeding-deterrent quinones, N-ethyl maleimide, and glutathione yielded data which supported our other experimental findings about the energy-transduction mechanism.

The reaction of glutathione with 1,4-naphthoquinone resulted in the half-wave potential of the quinone wave being shifted toward the right 0·025 V (Fig. 5). Such a small, but real, shift in potential indicated that an energy transfer and/or new compound had resulted between the chemicals in the solution. In the presence of glutathione, an anodic wave for 1,4-naphthoquinone appeared (Fig. 5); whereas, this quinone alone yielded only a cathodic wave (Fig. 5). This indicated that quinone had been reduced during its interaction with glutathione, and that

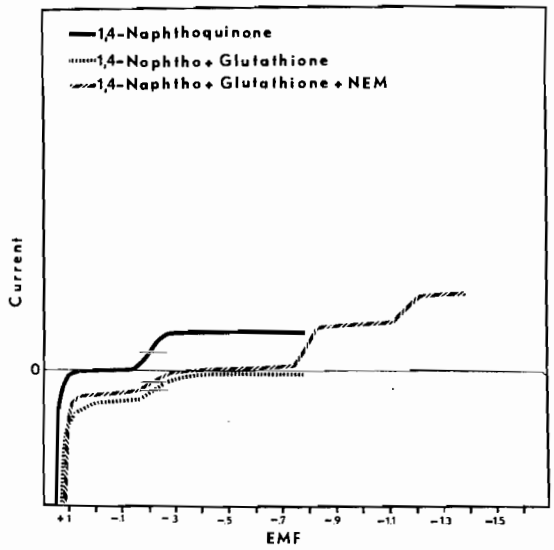

FIG. 5. Polarograms of 1,4-naphthoquinone, 1,4-naphthoquinone + glutathione, and 1,4-naphthoquinone + glutathione + N-ethyl maleimide (NEM), at 20 μA full scale sensitivity and a damping setting of 4. All concentrations of tested chemicals were 5×10^{-4} M, and the buffer used was 75 ml of phosphate buffer (pH 7) + 25 ml of 95% ethanol. Note the shift in half-wave potential of the wave for the 1,4-naphthoquinone when mixed with glutathione or glutathione + NEM. Changes in the diffusion current of the 1,4-naphthoquinone wave attributable to treatment also are shown.

the reduced substance subsequently was being oxidized at the dropping-mercury electrode.

Changes in the diffusion current observed with quinone alone versus quinone + glutathione indicated that complexing occurred. At a given molar concentration, the diffusion current of different chemicals should vary as the square root of their molecular weight. Glutathione + 1,4-naphthoquinone gave an anodic wave current for the quinone which was approximately equal to the $\sqrt{3}$ of the cathodic current for 1,4-naphthoquinone alone (Fig. 5). This would suggest that the new moiety was about three times the weight of the quinone alone. The combined molecular weight of 1,4-naphthoquinone + glutathione is 465 as compared to 158 for the quinone alone. These total findings would strongly support the existence of a complex between 1,4-naphthoquinone and glutathione.

Upon addition of the specific sulphhydryl group inhibitor, N-ethyl maleimide, to the mixture of 1,4-naphthoquinone and glutathione, the diffusion current for the 1,4-naphthoquinone wave fell between that obtained when the quinone was alone, and when it was combined with just glutathione (Fig. 5). These results would seem to indicate that the addition of N-ethyl maleimide reduced the amount of 1,4-naphthoquinone which was complexed with glutathione. These data thus would indicate some competition between the quinone and N-ethyl maleimide for glutathione and would especially implicate thiols as the important reaction site of glutathione.

Our findings regarding this energy-transduction mechanism in quinone inhibition of insect feeding would indicate that energy transfer between sulphhydryl groups and quinones provides a mechanism for the change in conformation of receptor macromolecules which could then allow altered inorganic ion flows to generate a characteristic action potential in the neuron. This information would seem to have significant usefulness in interpreting other reactions at membranes and other sites in living systems.

The extensive review of quinones as metabolic inhibitors by WEBB (1966) clearly indicated the broad occurrence and multiple effects of quinones in living organisms, and at the same time concluded that, in no case, had a potent biological action of a quinone been correlated with a specific mechanism. Webb further commented that ultimate clarification of such mechanisms of quinone action would likely provide useful information on the energy-transfer processes of such subjects as mitosis and cell differentiation.

REFERENCES

AMOORE J. E. (1965) Psychophysics of odor. *Cold Spr. Harb. Symp. quant. Biol.* **30**, 623–637.

BORG T. K. and NORRIS D. M. (1970) The ultrastructure of the sensory receptors of the antennae of *Scolytus multistriatus*. *Z. Zell. Mikr. Anat.* In press.

BORG T. K. and NORRIS D. M. (1971) The morphology of the sensory receptors of the American cockroach. *J. Morph.* In press.

DASTOLI F. R. and PRICE S. (1966) Sweet-sensitive protein from bovine taste buds: isolation and assay. *Science, Wash.* **154**, 905–907.

De Robertis E., Pellegrino de Iraldi A., Rodriquez de Lores Arnaiz G., and Gomez C. J. (1961) On the isolation of nerve endings and synaptic vesicles. *J. Biophys. Biochem. Cytol.* **9**, 229–235.

Donovan J. W. (1969) Changes in ultraviolet absorption produced by alteration of protein conformation. *J. biol. Chem.* **244**, 1961–1967.

Lowry O. H., Rosebrough N. J., Farr A. L., and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.

Morrison M., Steele W., and Danner D. J. (1969) The reaction of benzoquinone with amines and proteins. *Archs Biochem. Biophys.* **134**, 515–523.

Morton R. A. (1965) *Biochemistry of Quinones*. Academic Press, New York.

Norris D. M. (1969) Transduction mechanism in olfaction and gustation. *Nature, Lond.* **222**, 1263–1264.

Norris D. M. (1970) Quinol stimulation and quinone deterrency of gustation by *Scolytus multistriatus*. *Ann. ent. Soc. Am.* **63**, 476–478.

Pisano J. J. (1969) Peptides. In *Handbook of Neurochemistry* (Ed. by Lajtha A.), pp. 53–74. Plenum Press, New York.

Roth L. M. and Willis E. R. (1950) A study of cockroach behavior. *Am. Mid. Nat.* **47**, 66–129.

Szent-Györgyi A. (1960) *Introduction to a Submolecular Biology*. Academic Press, New York.

Telford J. N. and Matsumura F. (1970) ^{14}C-Dieldrin binding in subcellular nerve components of cockroaches. An autoradiographic and electron microscopic study. *J. econ. Ent.* In press.

Webb J. L. (1966) *Enzyme and Metabolic Inhibitors* **3**, pp. 421–594. Academic Press, New York.